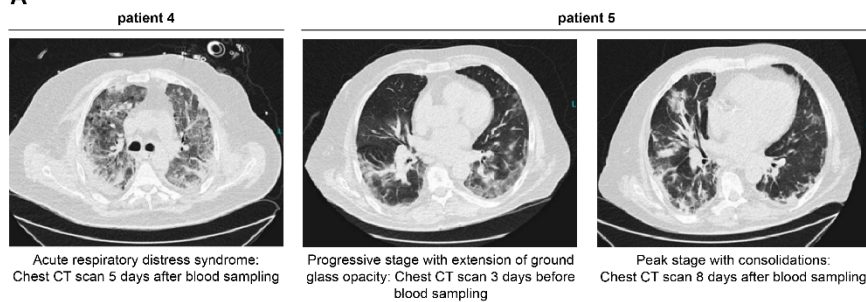


Supplementary information for Theobald et al., “Long-lived macrophage reprogramming drives spike protein-mediated inflammasome activation in COVID-19”

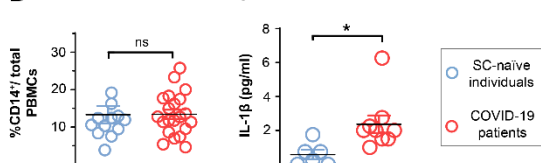
Appendix Figure S1 – S3	Additional data	Page 1-5
Appendix Table S1	Characteristics of blood donors for our study	Page 6-10
Appendix Table S2	Statistical data	Page 11-13

Appendix Figure S1

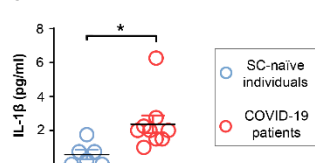
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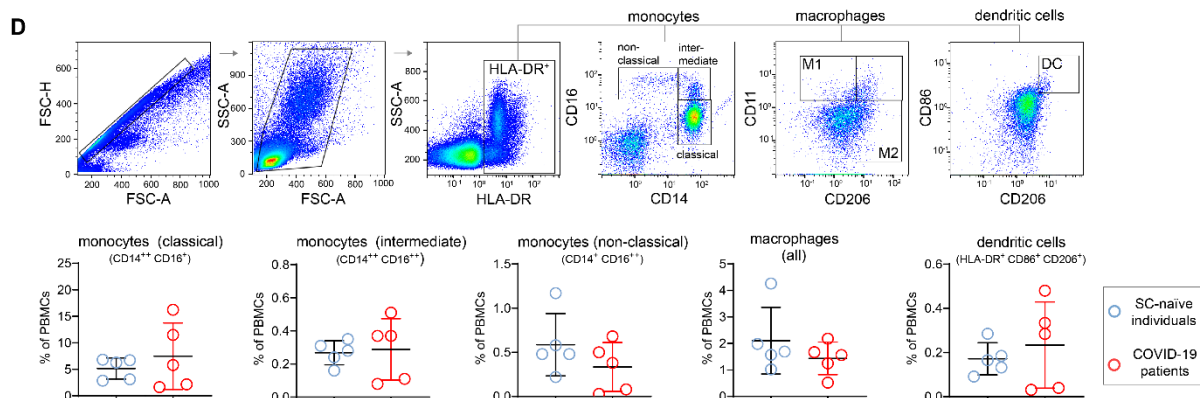
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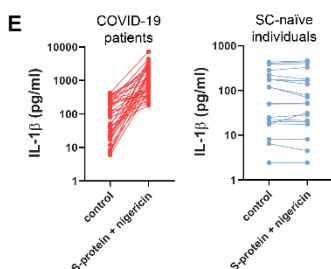
C



D

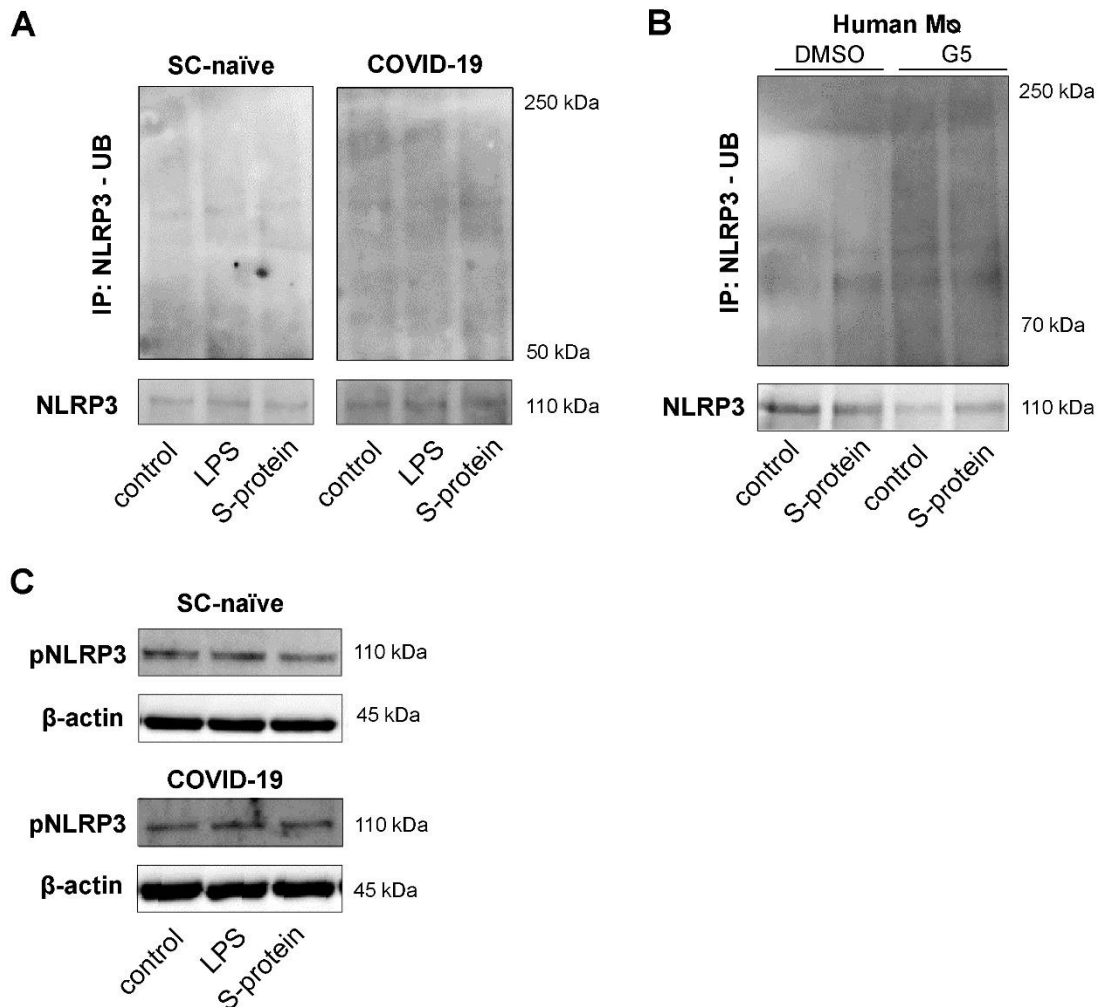


E



Appendix Figure S1: **A** Chest CT scan of patient 4 and 5 (see Supplementary tables 1) showing different pulmonary manifestations of disease. **B** Percentage of CD14⁺ cells per number of total PBMC calculated after CD14 magnetic bead selection for healthy individuals (n=13, blue) and COVID-19 patients (n=18, red). Standard deviation of the mean is indicated. **C** Cytometric Bead Array-based quantification of IL-1 β in plasma of COVID-19 patients (n = 6; red circles) or healthy individuals (n = 6; blue circles). For S1b and S1c students T-test with Welch correction were performed and significances are indicated. **D** Representative gating strategy (top panel) showing flow cytometry analysis for PBMC phenotyping regarding monocytes (CD16, CD14), macrophages (CD11c, CD206) and dendritic cells (CD86, CD206). Quantifications (bottom panel, % of total PBMC) of classical monocytes (CD14⁺⁺/CD16⁺), intermediate monocytes (CD14⁺⁺/CD16⁺⁺), non-classical monocytes (CD14⁺/CD16⁺⁺), macrophages (M1+M1/2+M2, see gating example) and dendritic cells (HLA-DR⁺/CD86⁺/CD206⁺) comparing healthy controls (blue) versus COVID-19 patients (red) (n=5 for healthy individuals and COVID-19 patients). **E** Quantification of IL-1 β concentration (pg/ml) in the supernatants of primary macrophage cultures from COVID-19 patients (n = 44; red bars) or healthy individuals (SC-naïve; blue) after stimulation with S-protein (0.1 μ g/ml) and nigericin subsequently. Control cells were left untreated. (log scale).

Appendix Figure S2

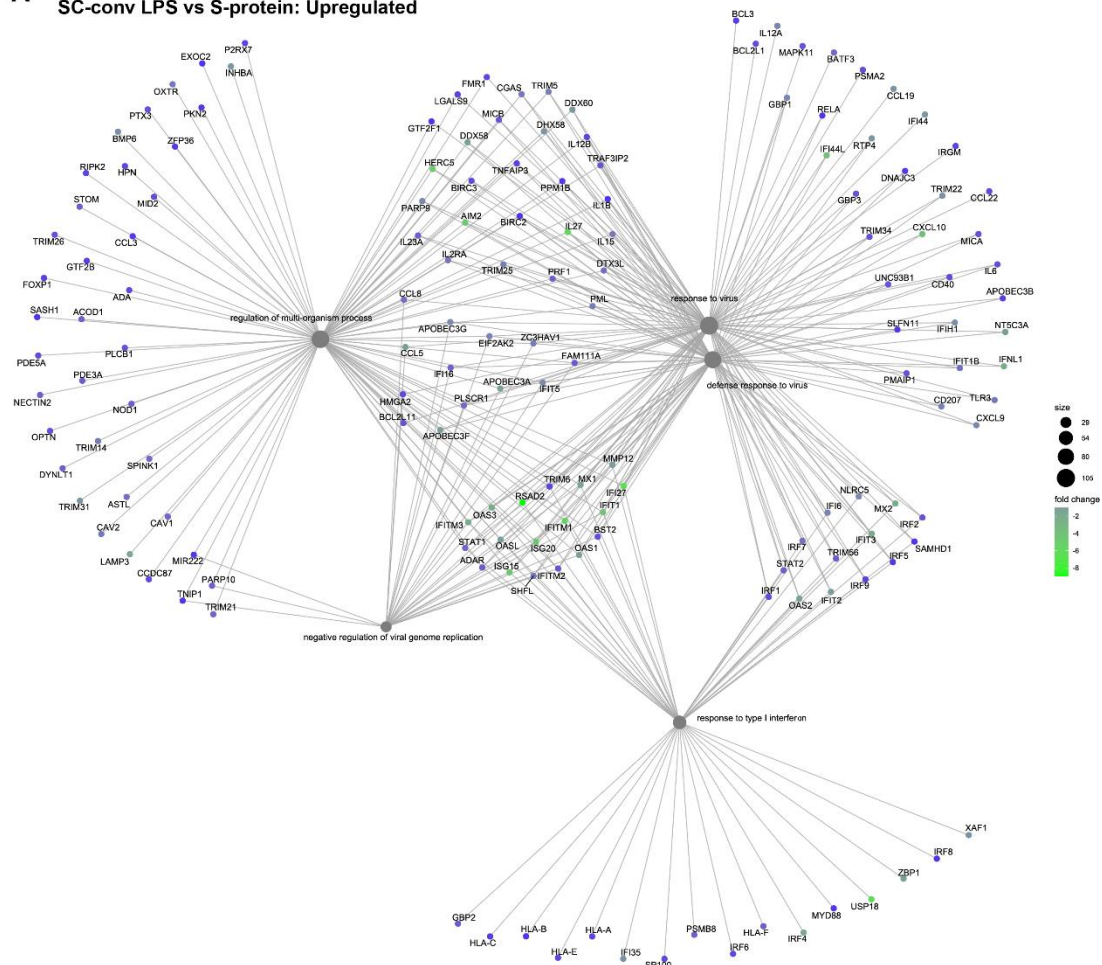


Appendix Figure S2: A Detection of upiquitinated NLRP3 in cell lysates from healthy (left) and COVID-19 (right) macrophages. Macrophages were stimulated with LPS or S-protein (4h) followed by 2h of nigericin treatment. Control cells were left unstimulated. NLRP3 (bottom) was detected in order to verify immune precipitation of the correct protein. Representative example of three individual experiments. **B** Detection of upiquitinated NLRP3 (top) total cell lysates from primary macrophages. Macrophages were stimulated with S-protein (4h) followed by 2h of nigericin treatment. 15min prior to nigericin the ubiquitinase isopaptidase inhibitor G5 (2 μ M) was added. Control cells were left unstimulated. NLRP3 (bottom) was detected in order to verify immune precipitation of the correct protein. Representative example of two individual experiments. Ubiquitin Antibody detects ubiquitin, polyubiquitin and ubiquitinated proteins (1:1000 dilution). **C** Detection of p-NLRP3 (110kDA, top row, antibody targets NLRP3 around the phosphorylation site of Ser295, dilution 1:1000) in total cell lysates

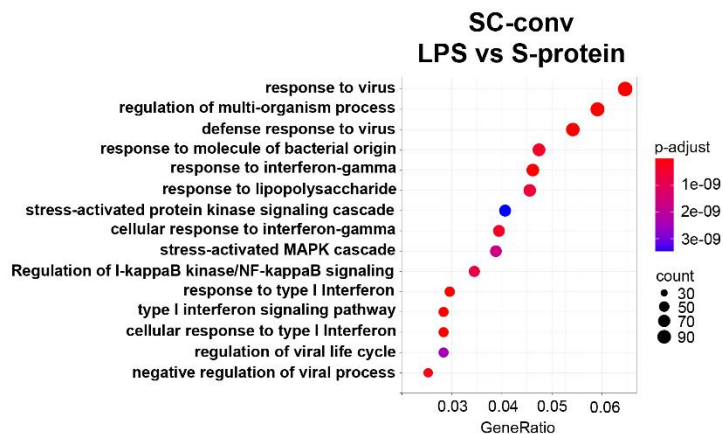
from healthy (upper panel) and COVID-19 (lower panel) macrophages. Macrophages were stimulated with LPS or S-protein (4h) followed by 2h of nigericin treatment. Control cells were left unstimulated and β -actin (45kDA) was used as loading control. Representative example of three individual experiments.

Appendix Figure S3

A SC-conv LPS vs S-protein: Upregulated



B



Appendix Figure S3: A Gene-concept network analysis, showing upregulated signaling pathways in LPS stimulated macrophages compared to S-protein stimulated macrophages in COVID-19 patient-derived cells (SC-conv). **B** Gene enrichment analysis indicating pathways activated in COVID-19 macrophages (SC-conv) stimulated LPS or S-Protein. Padj values are indicated (color code) and size of the circles corresponds to the number of DEGs (count). RNA-Seq analyses included data with padj <0.05 and log₂-fold change of ≤ -1 and ≥ 1 .

Appendix Table S1: characteristics of blood donors for our study.

A

COVID-19 patients (severe disease/hospitalized)												
SID	sex	age	O ₂ therapy	comorbidites	sampling after dx (days)	Days of hospitalization	COVID-19 treatment	Antibiotic treatment	CRP (mg/ml)	ferritin (µg/ml)	ANC (x10 ⁹ /l)	ALC (x10 ⁹ /l)
1	f	66	mask	myeloma	10	15	-	+	42	6585	1.4	0.4
2	m	52	mask	CKD, RA	6	12	-	+	95	3134	4.3	0.5
3	m	63	MV	HTN	18	22	-	+	126	1658	6.1	0.5
4	m	52	MV	none	15	43	-	+	247	6581	3.3	0.3
5	m	73	mask	AF, HTN, T2D	3	23	-	+	203	4542	2.5	0.5
6	m	57	mask	NASH	14	9	-	-	35	1901	3.6	1.6
7	f	50	mask	HIV, T2D	3	8	+ ¹	-	85	656	4.3	0.9
8	f	45	mask	none	3	9	-	-	97	1352	5.2	1.1
9	f	54	MV	HTN	42	45	+ ²	+	16	N/A	2.1	1.6
10	m	69	mask	CLL, HTN	36	56	+ ³	+	3	N/A	9.7	0.6
11	f	75	mask	AF, HTN	9	12	+ ⁴	+	5	N/A	11.7	4.1
12	m	60	mask	NSCLC, AF	8	8	+ ⁴	+	252	7420	24.3	1.40
13	f	62	mask	HTN	4	7	+ ⁴	-	40	341	8.8	1.3
14	m	53	MV	CHD, HTN, T2D	14	25	+ ⁴	+	82	562	5.3	1.7
15	f	53	mask	HT	3	5	+ ⁴	-	18	403	3.2	1.6
16	m	79	mask	CKD, HTN, T2D	2	26	+ ⁴	+	91	483	4.8	1.0
17	m	77	mask	COPD, AF, CKD	17	14	+ ⁴	-	2.4	N/A	10.1	2.5
18	m	43	-	HIV	32	15	-	-	1.2	N/A	1.8	1.9

19	m	86	MV	CHD, HTN, HT	2	N/A	+ ⁴	+	27	226	11.2	1.1
20	m	77	mask	CKD, T2D, CHD	3	19	+ ⁴	+	142	916	3.0	0.4
21	m	57	mask	NSCLC, T2D	2	9	+ ⁴	-	32	1270	2.4	0.9
22	f	82	mask	COPD, HTN, CKD	5	6	+ ⁴	+	78	140	81	0.7
23	m	55	MV	DLBCL, HTN	6	26	+ ⁴	+	114	806	3.3	0.2

B

COVID-19 patients (mild disease/outpatients) used for Fig. 3A												
SID	sex	age	O ₂ therapy	comorbidites	sampling after dx (days)	Days of hospitalization	COVID-19 treatment	Antibiotic treatment	CRP (mg/ml)	ferritin (µg/ml)	ANC (x10 ⁹ /l)	ALC (x10 ⁹ /l)
24	f	39	-	none	18	0	-	-	N/A	N/A	N/A	N/A
25	m	30	-	none	18	0	-	-	N/A	N/A	N/A	N/A
26	m	26	-	none	24	0	-	-	N/A	N/A	N/A	N/A
27	f	26	-	none	23	0	-	-	N/A	N/A	N/A	N/A
28	m	50	-	none	59	0	-	-	N/A	N/A	N/A	N/A
29	m	66	-	HTN	15	0	-	-	N/A	N/A	N/A	N/A
30	f	52	-	BA	61	0	-	-	N/A	N/A	N/A	N/A
31	m	19	-	T1D, BA	57	0	-	-	N/A	N/A	N/A	N/A
32	f	22	-	none	57	0	-	-	N/A	N/A	N/A	N/A
33	f	55	-	HTN	66	0	-	-	N/A	N/A	N/A	N/A
34	m	51	-	HTN, BA	65	0	-	-	N/A	N/A	N/A	N/A
35	m	28	-	none	20	0	-	-	N/A	N/A	N/A	N/A
36	m	28	-	none	22	0	-	-	N/A	N/A	N/A	N/A
37	m	24	-	none	28	0	-	-	N/A	N/A	N/A	N/A
38	m	23	-	none	18	0	-	-	N/A	N/A	N/A	N/A

39	m	29	-	none	30	0	-	-	N/A	N/A	N/A	N/A
40	f	27	-	none	30	0	-	-	N/A	N/A	N/A	N/A
41	f	18	-	none	32	0	-	-	N/A	N/A	N/A	N/A
42	f	18	-	none	32	0	-	-	N/A	N/A	N/A	N/A
43	f	31	-	none		35	-	-	N/A	N/A	N/A	N/A
44	m	41	-	none		27	-	-	N/A	N/A	N/A	N/A

C

Convalescent COVID-19 patients (Gene expression analysis)													
SID	sex	age	O₂ therapy	comorbidites	sampling after dx (days)	Days of hospitalization	COVID-19 treatment	Antibiotic treatment	CRP (mg/ml)	ferritin (µg/ml)	ANC (x10⁹/l)	ALC (x10⁹/l)	SARS- CoV2 IgG
45	m	67	-	HTN	24	0	-	-	0.6	134	2.18	1.70	+
46	m	21	-	none	32	0	-	-	7.7	327	4.94	3.14	+
47	f	34	-	none	49	0	-	-	0.6	66	2.28	1.65	+
48	m	31	-	none	40	0	-	-	0.6	50	3.62	2.62	+

D

Healthy individuals													
SID	sex	age		comorbidites					CRP (mg/ml)	ferritin (µg/ml)	ANC (x10 ⁹ /l)	ALC (x10 ⁹ /l)	SARS-CoV2 IgG
49	f	30		none					N/A	N/A	N/A	N/A	N/A
50	m	41		none					N/A	N/A	N/A	N/A	N/A
51	m	33		none					N/A	N/A	N/A	N/A	N/A
52	m	26		none					N/A	N/A	N/A	N/A	N/A
53	m	26		none					N/A	N/A	N/A	N/A	N/A
54	m	26		none					N/A	N/A	N/A	N/A	N/A
55	f	52*		BA					0.7	61	4.82	1.64	-
56	m	22*		none					1.4	138	2.74	2.09	-
57	f	62*		HTN					0.9	297	2.63	1.69	-
58	f	54		none					N/A	N/A	N/A	N/A	N/A
59	f	60		BA					N/A	N/A	N/A	N/A	N/A
60	f	47		none					N/A	N/A	N/A	N/A	N/A
61	m	49		none					N/A	N/A	N/A	N/A	N/A
62	m	45*		none					0.6	164	2.66	1.88	-
63	f	31		none					N/A	N/A	N/A	N/A	N/A
64	m	35		none					N/A	N/A	N/A	N/A	N/A
65	f	24		none					N/A	N/A	N/A	N/A	N/A
66	f	26		none					N/A	N/A	N/A	N/A	N/A
67	f	37		none					N/A	N/A	N/A	N/A	N/A

68	m	33		none					N/A	N/A	N/A	N/A	N/A
69	m	27		none					N/A	N/A	N/A	N/A	N/A
70	f	25		none					N/A	N/A	N/A	N/A	N/A
71	m	28		none					N/A	N/A	N/A	N/A	N/A
72	f	23		none					N/A	N/A	N/A	N/A	N/A

E

Tuberculosis patients										
SID	sex	age	Organ involvement	sampling after dx (days)	Antibiotic treatment	CRP (mg/ml)	ferritin (µg/ml)	ANC (x10 ⁹ /l)	ALC (x10 ⁹ /l)	
73	m	38	lymph nodes (cervical, paratracheal), sternum	19/469	+	4.1/1.1	N/A	5.1/5.7	1.2/1.4	
74	m	28	multiple bone lesions	4/551	+	47.6/0.6	N/A	5.9/4.4	1.3/1.7	
76	m	25	peritoneum, lymph nodes (abdominal), ascites, iliitis, soft tissue	5/461	+	226/0.9	N/A	5.2/4.1	0.8/1.5	
77	m	35	multiple lymph nodes (cervical, abdominal, mediastinum)	5/459	+	15.7/5.5	N/A	2.0/1.8	3.8/1.9	
78	m	53	soft tissue, bones (multiple lesions)	9/371	+	69/15.1	N/A	6.8/7.1	0.8/2.3	
79	m	50	pleura, lymph nodes	10/287	+	58.4/0.9	N/A	6.5/2.5	1/0.7	

AF: atrial fibrillation; ANC: absolute neutrophil count; ALC: absolute lymphocyte count; BA: bronchial asthma; CHD: chronic heart disease; CKD: chronic kidney disease; CLL: chronic lymphocytic leukemia; COPD: chronic obstructive pulmonary disease; CRP: c-reactive protein; DLBCL: diffuse large B cell lymphoma; dx: diagnosis; HIV: human immunodeficiency virus infection; HT: hypothyroidism; HTN: hypertension; MV: mechanical ventilation; N/A: not available; NASH: Non-Alcoholic steatohepatitis; NSCLC: non-small cell lung carcinoma; RA: rheumatoid arthritis; T1D: Type 1 diabetes; T2D: Type 2 diabetes; + yes/positive; - none/negative; * Samples were used for gene expression analysis. ¹Tocilizumab/Placebo (Study); ²Ribavirin/Lopinavir/Ritonavir; ³Convalescent plasma therapy; ⁴Remdesivir/Dexamethason

Appendix Table S2: Statistical data

Statistical data			
Figure	Statistical test	Condition	p-value
1D	Two-way ANOVA Tukey's multiple comparison test	Control SC-naïve vs LPS + nigericin SC-naïve	0.0050
		Control SC-naïve vs LPS + nigericin COVID-19	<0.0001
		Control SC-naïve vs S-protein + nigericin COVID-19	<0.0001
		LPS + nigericin SC-naïve vs LPS + nigericin COVID-19	0.0031
		S-protein + nigericin SC-naïve vs S-protein + nigericin COVID-19	<0.0001
1E	Two-way ANOVA with Tukey's multiple comparison test	Control SC-naïve vs LPS SC-naïve	<0.0001
		Control COVID-19 vs LPS COVID-19	<0.0001
		Control SC-naïve vs LPS + nigericin SC-naïve	<0.0001
		Control COVID-19 vs LPS + nigericin COVID-19	<0.0001
		Control SC-naïve vs S-protein SC-naïve	0.0029
		Control COVID-19 vs S-protein COVID-19	0.0023
		Control SC-naïve vs S-protein + nigericin SC-naïve	0.0015
		Control COVID-19 vs S-protein + nigericin COVID-19	0.0019
2A	One-way ANOVA with Tukey's multiple comparison test	DMSO vs MCC950	<0.0001
		DMSO vs VX-765	0.0010
2E	Kolmogorov-Smirnov test	Control SC-naïve vs S-protein COVID-19	0.0079
		Control COVID-19 vs S-protein COVID-19	0.0159
		S-protein SC-naïve vs S-protein COVID-19	0.0357
3C	Unpaired, two tailed t-test	SC-naïve vs SC-conv	0.043
4A	Unpaired, two tailed t-test	SC-naïve vs SC-conv	0.0332
4B	One-way ANOVA	DMSO vs KINK-1	0.0037

		DMSO vs MMG-11	0.0005
4C	One-way ANOVA	DMSO vs KINK	0.0019
4D	Two-way ANOVA with Tukey's multiple comparison test	Unstimulated SC-naïve vs DMSO SC-naïve	0.0447
		Unstimulated COVID-19 vs DMSO COVID19	<0.0001
		DMSO COVID-19 vs KINK-1 COVID19	<0.0001
		DMSO COVID-19 vs MMG-11 COVID19	<0.0001
4E	One-way ANOVA	1 ng/ml vs 10 µg/ml	0.0228
5E	Brown-Forsythe and Welsh ANOVA test	Unstimulated vs LPS + nigericin	0.0415
		Unstimulated vs FCCP + nigericin	0.0179
		Unstimulated vs H ₂ O ₂ + nigericin	0.0247
		S-protein + nigericin vs FCCP + nigericin	0.0190
		S-protein + nigericin vs H ₂ O ₂ + nigericin	0.0252
5E	Two-way ANOVA with Tukey's multiple comparison test	Control healthy vs LPS healthy	
		Control acute tuberculosis vs LPS acute tuberculosis before Tx	0.0022
		Control acute tuberculosis vs LPS acute tuberculosis after Tx	0.0027
		Control acute tuberculosis vs S-protein acute tuberculosis before Tx	0.0011
		S-protein healthy vs S-protein acute tuberculosis before Tx	0.0305
EV1A	Unpaired, two tailed t-test with Welsh correction	SC-naïve vs COVID19	0.0351
EV1C	Two-way ANOVA with Tukey's multiple comparison test	ATP COVID-19 vs LPS + ATP COVID-19	0.0017
EV1D	One-way ANOVA	DMSO vs MCC950	<0.0001
		DMSO vs VX-765	0.0003
EV1F	Two-way ANOVA with Tukey's multiple comparison test	Control SC-naïve vs LPS SC-naïve	<0.0001
		Control SC-naïve vs S-protein SC-naïve	0.0002
		Control COVID-19 vs LPS COVID-19	<0.0001

		Control COVID-19 vs S-protein COVID-19	<0.0001
EV1J	One-way ANOVA	DMSO vs S-protein + nigericin	0.0198
EV1K	Two-way ANOVA with Tukey's multiple comparison test	S-protein COVID-19 vs S2-domain COVID-19	<0.0001